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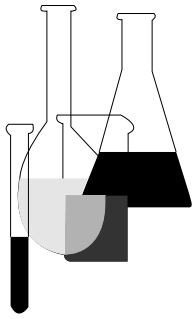
Health Effects Test Guidelines OPPTS 870.4300 Combined Chronic Toxicity/Carcinogenicity



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0132 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 870.4300 Combined chronic toxicity/carcinogenicity.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline are 40 CFR 798.3320 Combined Chronic Toxicity/Oncogenicity; OPP 83–5 Combined Chronic Toxicity/Oncogenicity (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982; and OECD 453 Combined Chronic Toxicity/Carcinogenicity Studies.

(b) **Purpose.** The objective of a combined chronic toxicity/carcinogenicity study is to determine the effects of a substance in a mammalian species following prolonged and repeated exposure. The application of this guideline should generate data which identify the majority of chronic and carcinogenicity effects and determine dose-response relationships. The design and conduct should allow for the detection of neoplastic effects and a determination of the carcinogenic potential as well as general toxicity, including neurological, physiological, biochemical, and hematological effects and exposure-related morphological (pathology) effects.

(c) **Definitions.** The definitions in section 3 of TSCA and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this guideline. The following definitions also apply to this guideline.

Carcinogenicity is the development of neoplastic lesions as a result of the repeated daily exposure of experimental animals to a chemical by the oral, dermal, or inhalation routes of exposure.

Chronic toxicity is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by the oral, dermal, or inhalation routes of exposure.

Cumulative toxicity is the adverse effects of repeated dose occurring as a result of prolonged action on, or increased concentration of, the administered test substance or its metabolites in susceptible tissues.

Dose in a combined chronic toxicity/carcinogenicity is the amount of test substance administered via the oral, dermal, or inhalation routes for a period of up to 24 months. Dose is expressed as weight of the test substance (grams, milligrams) or as weight of the test substance per unit body weight of test animal (milligrams per kilogram), or as weight of the test substance in parts per million (ppm) in food or drinking water. When exposed via inhalation, dose is expressed as weight of the test substance per unit volume of air (milligrams per liter) or as parts per million.

No-observed-effect-level (NOEL) is the maximum dose used in a study which produces no observed adverse effects. The NOEL is usually expressed in terms of the weight of a test substance given daily per unit weight of test animal (milligrams per kilogram per day).

Target organ is any organ of a test animal showing evidence of an effect induced by a test substance.

(d) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** Preliminary studies providing data on acute, subchronic, and metabolic responses should have been carried out to permit an appropriate choice of animals (species and strain). As discussed in other guidelines, the mouse and rat have been most widely used for assessment of carcinogenic potential, while the rat and dog have been most often studied for chronic toxicity. For the combined chronic toxicity/carcinogenicity study via the oral and inhalation routes, the rat is the species of choice and for the dermal route, the mouse is species of choice. If other species are used, the tester should provide justification/reasoning for their selection. The strain selected should be susceptible to the carcinogenic or toxic effect of the class of substances being tested, if known, and provided it does not have a spontaneous background incidence too high for meaningful assessment. Commonly used laboratory strains should be employed.

(ii) **Age/weight.** (A) Testing should be started with young healthy animals as soon as possible after weaning and acclimatization.

(B) Dosing should generally begin no later than 8 weeks of age.

(C) At commencement of the study, the weight variation of animals used should not exceed ± 20 percent of the mean weight for each sex.

(D) Studies using prenatal or neonatal animals may be recommended under special conditions.

(iii) **Sex** (A) Equal numbers of animals of each sex should be used at each dose level.

(B) Females should be nulliparous and nonpregnant.

(iv) **Numbers.** (A) At least 100 rodents (50 males and 50 females) should be used at each dose level and concurrent control group. At least 20 additional rodents (10 males and 10 females) should be used for satellite dose groups and the satellite control group. The purpose of the satellite group is to allow for the evaluation of pathology other than neoplasia.

(B) For a meaningful and valid statistical evaluation of long term exposure and for a valid interpretation of negative results, the number of animals in any group should not fall below 50 percent at 15 months in

mice and 18 months in rats. Survival in any group should not fall below 25 percent at 18 months in mice and 24 months in rats.

(C) To avoid bias, the use of adequate randomization procedures for the proper allocation of animals to test and control groups is required.

(D) Each animal should be assigned a unique identification number. Dead animals (and their preserved organs) and tissues, and microscopic slides should be identified by reference to the unique numbers assigned.

(v) **Husbandry.** (A) Animals may be group-caged by sex, but the number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g., morbidity, excitability) may indicate a need for individual caging. Rodents should be housed individually in dermal studies and during exposure in inhalation studies.

(B) The temperature of the experimental animal rooms should be at 22 ± 3 °C.

(C) The relative humidity of the experimental animal rooms should be 30 to 70 percent.

(D) Where lighting is artificial, the sequence should be 12 h light/ 12 h dark.

(E) Control and test animals should be fed from the same batch and lot. The feed should be analyzed to assure uniform distribution and adequacy of nutritional requirements of the species tested and for impurities that might influence the outcome of the test. Animals should be fed and watered ad libitum with food replaced at least weekly.

(F) The study should not be initiated until animals have been allowed a period of acclimatization/quarantine to environmental conditions, nor should animals from outside sources be placed on test without an adequate period of quarantine.

(2) **Control and test substances** (i) Where necessary, the test substance is dissolved or suspended in a suitable vehicle. If a vehicle or diluent is needed, it should not elicit toxic effects itself. It is recommended that wherever possible the usage of an aqueous solution be considered first, followed by consideration of a solution in oil, and finally solution in other vehicles.

(ii) One lot of the test substance should be used throughout the duration of the study if possible, and the research sample should be stored under conditions that maintain its purity and stability. Prior to the initiation of the study, there should be a characterization of the test substance, including the purity of the test compound, and, if possible, the name and quantities of contaminants and impurities.

(iii) If the test or control substance is to be incorporated into feed or another vehicle, the period during which the test substance is stable in such a mixture should be determined prior to the initiation of the study. Its homogeneity and concentration should be determined prior to the initiation of the study and periodically during the study. Statistically randomized samples of the mixture should be analyzed to ensure that proper mixing, formulation, and storage procedures are being followed, and that the appropriate concentration of the test or control substance is contained in the mixture.

(3) **Control groups.** A concurrent control group (50 males and 50 females) and a satellite control group (10 males and 10 females) are required. These groups should be untreated or if a vehicle is used in administering the test substance, vehicle control groups. If the toxic properties of the vehicle are not known, both untreated and vehicle control groups are required. Animals in the satellite control group should be sacrificed at the same time the satellite test group is terminated.

(4) **Dose levels and dose selection.** (i) For risk assessment purposes, at least three dose levels should be used, in addition to the concurrent control group. Dose levels should be spaced to produce a gradation of effects. A rationale for the doses selected must be provided.

(ii) The highest dose level in rodents should elicit signs of toxicity without substantially altering the normal life span due to effects other than tumors. The highest dose should be determined based on the findings from a 90–day study to ensure that the dose used is adequate to asses the chronic toxicity and the carcinogenic potential of the test substance. Thus, the selection of the highest dose to be tested is dependent upon changes observed in several toxicological parameters in subchronic studies. The highest dose tested need not exceed 1,000 mg/kg/day.

(iii) The intermediate dose levelss should be spaced to produce a gradation of toxic effects.

(iv) The lowest dose level should produce no evidence of toxicity.

(v) For chronic toxicity assessment, an additional treated and a concurrent control satellite group may be included in the study. The highest dose for satellite animals should be chosen to produce frank toxicity, but not excessive lethality, in order to elucidate a toxicological profile of the test substance.

(vi) For skin carcinogenicity studies, when toxicity to the skin is a determining factor, the highest dose selected should not destroy the functional integrity of the skin, the intermediate doses should be a minimally irritating dose and the low dose should be the highest nonirritating dose.

(vii) The criteria for selecting the dose levels for skin carcinogenicity studies, based on gross and histopathologic dermal lesions, are as follows:

(A) Gross criteria for reaching the high dose:

(*1*) Erythema (moderate).

(*2*) Scaling.

(*3*) Edema (mild).

(*4*) Alopecia.

(*5*) Thickening.

(B) Histologic criteria for reaching the high-dose:

(*1*) Epidermal hyperplasia.

(*2*) Epidermal hyperkeratosis.

(*3*) Epidermal parakeratosis.

(*4*) Adnexal atrophy/hyperplasia.

(*5*) Fibrosis.

(*6*) Spongiosis (minimal-mild).

(*7*) Epidermal edema (minimal-mild).

(*8*) Dermal edema (minimal-moderate).

(*9*) Inflammation (moderate).

(C) Gross criteria for exceeding the high-dose:

(*1*) Ulcers-fissures, exudate/crust (eschar),nonviable (dead) tissues.

(*2*) Anything leading to destruction of the functional integrity of the epidermis (e.g., caking, fissuring, open sores, eschar).

(D) Histologic criteria for exceeding the high-dose:

(*1*) Epidermal hyperplasia.

(*2*) Epidermal hyperkeratosis.

(*3*) Epidermal parakeratosis.

(*4*) Adnexal atrophy/hyperplasia.

(*5*) Fibrosis.

(*6*) Spongiosis (minimal-mild).

(*7*) Epidermal edema (minimal-mild).

(*8*) Dermal edema (minimal-moderate).

(*9*) Inflammation (moderate).

(*10*) Crust.

(*11*) Microulcer.

(*12*) Epidermal edema (moderate-marked).

(*13*) Dermal edema (marked).

(*14*) Inflammation (marked).

(5) **Administration of the test substance.** The three main routes of administration are oral, dermal, and inhalation. The choice of the route of administration depends upon the physical and chemical characteristics of the test substance and the form typifying exposure in humans.

(i) **Oral studies.** If the test substance is administered by gavage, the animals are dose with the test substance on a 7–day per week basis for a period of at least 18 months for mice and hamsters and 24 months for rats. However, based primarily on practical considerations, dosing by gavage on a 5–day per week basis in acceptable. If the test substance is administered via capsule, in the drinking water or mixed in the diet, then exposure should be on a 7–day per week basis.

(ii) **Dermal studies.** (A) The animals should be treated with the test substance, for at least 6 hours per day on a 7–day per week basis for a period of at least 18 months for mice and hamsters and 24 months for rats. However, based primarily on practical considerations, application on a 5–day per week basis is acceptable. Dosing should be conducted at approximately the same time each day.

(B) Fur should be clipped weekly from the dorsal area of the trunk of the test animals. Care should be taken to avoid abrading the skin which could alter its permeability. A minimum of 24 hours should be allowed for the skin to recover before the next dosing of the animal.

(C) The test substance should be applied uniformly over a shaved area which is approximately 10 percent of the total body surface area. In order to dose approximately 10 percent of the body surface, the area starting at the scapulae (shoulders) to the wing of the ileum (hipbone) and half way down the flank on each side of the animal should be shaved. The volume of application should be kept constant and should not exceed 100 μL for the mouse and 300 μL for the rat; different concentrations of the test solution should be prepared for different dose levels. With highly toxic substances, the surface area covered may be less, but as much

of the area as possible should be covered with as thin and uniform a film as practical. The test material is not removed after application.

(D) During the exposure period, the application site should not be covered when mice or hamsters are the species of choice. However, for rats, the test substance may be held, if necessary, in contact with the skin with a porous gauze dressing and nonirritating tape, if necessary. The test site should be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance.

(iii) **Inhalation studies.** (A) The animals should be exposed to the test substance, for 6 h/day on a 7–day per week basis, for a period of at least 18 months in mice and 24 months in rats. However, based primarily on practical considerations, exposure for 6 h/day on a 5–day per week basis is acceptable.

(B) The animals should be tested in dynamic inhalation equipment designed to sustain a minimum air flow of 10 air changes per hour, an adequate oxygen content of at least 19 percent, and uniform conditions throughout the exposure chamber. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into surrounding areas.

(C) The selection of a dynamic inhalation chamber should be appropriate for the test substance and test system. Where a whole body chamber is used, individual housing must be used to minimize crowding of the test animals and maximize their exposure to the test substance. To ensure stability of a chamber atmosphere, the total volume occupied by the test animals should not exceed 5 percent of the volume of the test chamber. It is recommended, but not required, that nose-only or head-only exposure be used for aerosol studies in order to minimize oral exposures due to animals licking compound off their fur. The animals should be acclimated and heat stress minimized.

(D) The temperature at which the test is performed should be maintained at 22 ± 2 °C. The relative humidity should be maintained between 30 to 70 percent, but in certain instances (e.g., tests of aerosols, use of water vehicle) this may not be practicable.

(E) The rate of air flow should be monitored continuously but recorded at least every 30 minutes.

(F) Temperature and humidity should be monitored continuously but should be recorded at least every 30 minutes.

(G) The actual concentrations of the test substance should be measured in the breathing zone. During the exposure period, the actual concentrations of the test substance should be held as constant as practicable,

monitored continuously or intermittently depending on the method of analysis, and recorded at least at the beginning, at an intermediate time, and at the end of the exposure period.

(H) During the development of the generating system, particle size analysis should be performed to establish the stability of aerosol concentrations with respect to particle size. During exposure, analysis should be conducted as often as necessary to determine the consistency of particle size distribution. The mass median aerodynamic diameter (MMAD) particle size range should be between 1–3 μm. The particle size of hygroscopic materials should be small enough to allow pulmonary deposition once the particles swell in the moist environment of the respiratory tract.

(I) Feed should be withheld during exposure. Water may also be withheld during exposure.

(6) **Observation period.** It is necessary that the duration of the chronic toxicity/carcinogenicity study comprise the majority of the normal life span of the strain of animals to be used. This time period should not be less than 24 months for rats and 18 months for mice, and ordinarily not longer than 30 months for rats and 24 months for mice. For longer time periods, and where any other species are used, consultation with the Agency in regard to the duration of the study is advised.

(7) **Observation of animals.** (i) Observations should be at least once each day for morbidity and mortality. Appropriate actions should be taken to minimize loss of animals from the study (e.g. necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(ii) A careful clinical examination should be made at least once weekly. Observations should be detailed and carefully recorded, preferably using explicitly defined scales. Observations should include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of motor activity, gait and posture, reactivity to handling or sensory stimuli, grip strength and stereotypies or bizarre behavior (e.g., self-mutilation, walking backwards).

(iii) Body weights should be recorded individually for all animals: Once a week during the first 13 weeks of the study and at least once every 4 weeks thereafter unless signs of clinical toxicity suggest more frequent weighing to facilitate monitoring of health status.

(iv) When the test substance is administered in the feed or drinking water, measurements of feed or water consumption, respectively, should be determined weekly during the first 13 weeks of the study and then

at approximately monthly intervals unless health status or body weight changes dictate otherwise.

(v) Moribund animals should be removed and sacrificed when noticed and the time of death should be recorded as precisely as possible. At the end of the study period, all survivors should be sacrificed.

(8) **Clinical pathology.** Hematology, clinical chemistry and urinalyses should be performed from 10 animals per sex per group. The parameters should be examined at approximately 6 month intervals during the conduct of the study and at termination. If possible, these collections should be from the same animals at each interval. If hematological and biochemical effects are seen in the subchronic study, testing should also be performed at 3 months.If clinical observations suggest deterioration in health of the animals during the study, a differential blood count of the affected animals should be performed.

(i) **Hematology.** The recommended parameters are: Hemoglobin and hematocrit concentrations, red blood cell count, white blood cell count, differential leukocyte count, platelet count and a measure of clotting potential, such as prothrombin time or thromboplastin time.

(ii) **Clinical chemistry.** Parameters which are considered appropriate to all studies are electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance and signs of clinical toxicity. Suggested blood clinical chemistry determinations are:

(A) Electrolytes.

(*1*) Calcium.

(*2*) Chloride.

(*3*) Magnesium.

(*4*) Phosphorous.

(*5*) Potassium.

(*6*) Sodium.

(B) Enzymes.

(*1*) Alkaline phosphatase.

(*2*) Alanine aminotransferase.

(*3*) Aspartate aminotransferase.

(*4*) Gamma glutamyl transferase.

(*5*) Sorbitol dehydrogenase.

(C) Other.

(*1*) Albumin.

(*2*) Blood creatinine.

(*3*) Blood urea nitrogen.

(*4*) Globulin.

(*5*) Glucose (fasting).

(*6*) Total Bilirubin.

(*7*) Total cholesterol.

(*8*) Total serum protein.

Other determinations which may be necessary for an adequate toxicological evaluation include analyses of lipids, hormones, acid/base balance, methemoglobin and cholinesterase. Additional clinical chemistry may be employed where necessary to extend the investigation of observed effects.

(iii) **Urinalyses.** The following determinations should be made from either individual animals or on a pooled sample per sex per group: Appearance (volume and specific gravity), protein, glucose, ketones, bilirubin, occult blood (semiquantitatively), and microscopy of sediment (semiquanti- tatively).

(9) **Optional immunotoxicity screen.** To fulfill, in part, requirements for an immunotoxicity screen, subpopulation of splenic or peripheral blood lymphocytes in the rodents should be enumerated and quantified. Total T-, Total B-, Total T-helper, T-suppressor/cytotoxic and natural killer (NK) cell populations should be determined on at least 10 rodents of each sex in each group at the end of the study.

(10) **Ophthalmological examination.** Examinations should be made **on all animals** using an ophthalmoscope or an equivalent device prior to the administration of the test substance and at termination of the study on 10 animals per sex in the high-dose and control groups. If changes in eyes are detected, all animals should be examined.

(11) **Gross necropsy.** (i) A complete gross examination should be performed on all animals, including those which died during the experiment or were killed in a moribund condition.

(ii) The liver, lungs, kidneys, brain, spleen, and gonads should be trimmed and weighed wet, as soon as possible after dissection to avoid

drying. The organs should be weighed from interim sacrifice animals as well as from at least 10 animals per sex per group at terminal sacrifice.

(iii) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination.

(A) Digestive system.

(*1*) Salivary glands.

(*2*) Esophagus.

(*3*) Stomach.

(*4*) Duodenum.

(*5*) Jejunum.

(*6*) Ileum.

(*7*) Cecum.

(*8*) Colon.

(*9*) Rectum.

(*10*) Liver.

(*11*) Pancreas.

(*12*) Gallbladder (mice).

(*13*) Bile duct (rat).

(B) Nervous system.

(*1*) Brain (multiple sections).

(*2*) Pituitary.

(*3*) Peripheral nerves.

(*4*) Spinal cord (three levels).

(*5*) Eyes (retina, optic nerve).

(C) Glandular system.

(*1*) Adrenals.

(*2*) Parathyroids.

(*3*) Thyroids.

(D) Respiratory system.

(*1*) Trachea.

(*2*) Lung.

(*3*) Pharynx.

(*4*) Larynx.

(*5*) Nose (inhalation studies only).

(E) Cardiovascular/hematopoietic system.

(*1*) Aorta (thoracic).

(*2*) Heart.

(*3*) Bone marrow.

(*4*) Lymph nodes.

(*5*) Spleen.

(*6*) Thymus.

(F) Urogenital system.

(*1*) Kidneys.

(*2*) Urinary bladder.

(*3*) Prostate.

(*4*) Testes/epididymides.

(*5*) Seminal vesicles.

(*6*) Uterus.

(*7*) Ovaries.

(G) Other.

(*1*) All gross lesions and masses.

(*2*) Sternum and/or femur.

(iv) In inhalation studies, the entire respiratory tract, including nose, pharynx, larynx, and paranasal sinuses should be examined and preserved. In dermal studies, skin from treated and adjacent control skin sites should be examined and preserved.

(v) Inflation of lungs and urinary bladder with a fixative is the optimal method for preservation of these tissues. The proper inflation and fixation

of the lungs in inhalation studies is essential for appropriate and valid histopathological examination.

(vi) Information from clinical pathology and other in-life data should be considered before microscopic examination, since these data may provide significant guidance to the pathologist.

(12) **Histopathology.** (i) The following histopathology should be performed:

(A) Full histopathology on the organs and tissues, listed under paragraph (d)(11)(iii) of this guideline of all animals in the control and high dose groups and of all animals that died or were killed during the study.

(B) All gross lesions in all animals.

(C) Target organs in all animals.

(D) Lungs, liver and kidneys of all animals. Special attention to examination of the lungs of rodents should be made for evidence of infection since this provides an assessment of the state of health of the animals.

(ii) If the results show substantial alteration of the animal's normal life span, the induction of effects that might affect a neoplastic response, or other effects that might compromise the significance of the data, the next lower levels should be examined fully as described above in paragraph (d)(12)(i) of this guideline.

(iii) An attempt should be made to correlate gross observations with microscopic findings.

(iv) Tissues and organs designated for microscopic examination should be fixed in 10 percent buffered formalin or a recognized suitable fixative as soon as necropsy is performed and no less than 48 hours prior to trimming. Tissues should be trimmed to a maximum thickness of 0.4 cm for processing.

(e) **Data and reporting**—(1) **Treatment of results.** (i) Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion.

(ii) All observed results, (quantitative and qualitative) should be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods including significance criteria should be selected during the design of the study.

(2) **Evaluation of study results.** (i) The findings of a combined chronic toxicity/carcinogenicity study should be evaluated in conjunction with the findings of previous studies and considered in terms of the toxic

effects, the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence, incidence and severity of abnormalities (including behavioral and clinical abnormalities), gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects.

(ii) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailablity of the test substance should be considered.

(iii) In order for a negative test to be acceptable, it should meet the following criteria—no more than 10 percent of any group is lost due to autolysis, cannibalism, or management problems, and survival in each group is no less than 50 percent at 15 months for mice and 18 months for rats. Survival should not fall below 25 percent at 18 months for mice and 24 months for rats.

(iv) The use of historical control data from an appropriate time period from the same testing laboratory (i.e, the incidence of tumors and other suspect lesions normally occurring under the same laboratory conditions and in the same strain of animals employed in the test) is helpful for assessing the significance of changes observed in the current study.

(3) **Test report.** (i) In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, 40 CFR part 160, and the OECD Principles of GLP ((ISBN 92–64–12367–9), the following specific information should be reported:

(A) Test substance characterization should include:

(*1*) Chemical identification.

(*2*) Lot or batch number.

(*3*) Physical properties.

(*4*) Purity/impurities.

(*5*) Identification and composition of any vehicle used.

(B) Test system should contain data on:

(*1*) Species and strain of animals used and rationale for selection if other than that recommended.

(*2*) Age including body weight data and sex.

(*3*) Test environment including cage conditions, ambient temperature, humidity, and light/dark periods.

(C) Test procedure should include the following data:

(*1*) Method of randomization used.

(*2*) Full description of experimental design and procedure.

(*3*) Dose regimen including levels, methods, and volume.

(4) **Test results.** (i) Group animal data. Tabulation of toxic response data by species, strain, sex and exposure level for:

(A) Number of animals exposed.

(B) Number of animals showing signs of toxicity.

(C) Number of animals dying.

(ii) Individual animal data. Data should be presented as summary (group mean) as well as for individual animals.

(A) Time of death during the study or whether animals survived to termination.

(B) Time of observation of each abnormal sign and its subsequent course.

(C) Body weight data.

(D) Feed and water consumption data, when collected.

(E) Results of ophthalmological examination, when performed.

(F) Results of hematological tests performed.

(G) Results of clinical chemistry tests performed.

(H) Results of urinalysis tests performed.

(I) Results of immunotoxicity screen, when performed.

(J) Necropsy findings including absolute/relative organ weight data.

(K) Detailed description of all histopathological findings.

(L) Statistical treatment of results where appropriate.

(M) Historical control data.

(iii) In addition, for inhalation studies the following should be reported:

(A) Test conditions. The following exposure conditions must be reported.

(*1*) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulates and aerosols, meth-

od of conditioning air, treatment of exhaust air and the method of housing the animals in a test chamber.

(*2*) The equipment for measuring temperature, humidity, and particulate aerosol concentrations and size should be described.

(B) Exposure data. These should be tabulated and presented with mean values and a measure of variability (e.g. standard deviation) and should include:

(*1*) Airflow rates through the inhalation equipment.

(*2*) Temperature and humidity of air.

(*3*) Actual (analytical or gravimetric) concentration in the breathing zone.

(*4*) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(*5*) Particle size distribution, and calculated mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

(*6*) Explanation as to why the desired chamber concentration and/or particle size could not be achieved (if applicable) and the efforts taken to comply with this aspect of the guidelines.

(f) **Quality assurance.** A system should be developed and maintained to assure and document adequate performance of laboratory staff and equipment. The study must be conducted in compliance with the GLP regulations as described by the Agency (40 CFR parts 160 and 792) and the OECD Principles of GLP (ISBN 92–64–12367–9).

(g) **References.** The following references should be consulted for additional background information on this guideline.

(1) Benitz, K.F. *Measurement of Chronic Toxicity.* Methods of Toxicology. Ed. G.E. Paget. Blackwell, Oxford. pp. 82–131 (1970).

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